Micellar Systems

~~The following specification is a Continuation-In-Part of United States Patent Application 09/354,957 filed on July 16th, 1999.~~ This application is a divisional of Application No. 10/081,461; filed February 21, 2002, which is a continuation-in-part of Application No. 09/354,957, filed July 16, 1999, issued as U.S. Patent 6,429,200, which claims the benefit of U.S. Provisional Application No. 60/093,321, filed 07/17/1998.

FEDERALLY SPONSORED RESEARCH

N/A

Field of the Invention

The invention generally relates to micellar systems for use in biologic systems. More particularly, a process is provided for the use of reverse micelles for the delivery of nucleic acids and genes to cells.

Background

Biologically active compounds such as proteins, enzymes, and nucleic acids have been delivered to the cells using amphipathic compounds that contain both hydrophobic and hydrophilic domains. Typically these amphipathic compounds are organized into vesicular structures such as liposomes, micellar, or inverse micellar structures. Liposomes can contain an aqueous volume that is entirely enclosed by a membrane composed of lipid molecules (usually phospholipids) (R.C. New, p. 1, chapter 1, "Introduction" in Liposomes: A Practical Approach, ed. R.C. New IRL Press at Oxford University Press, Oxford, 1990). Micelles and inverse micelles are microscopic vesicles that contain amphipathic molecules but do not contain an aqueous volume that is entirely enclosed by a

restricted to) polyoxyethylene alcohol's, polyoxyethylene isoalcohol, polyoxyethylene p-t-octyl phenol (Triton), polyoxyethylene nonylphenol, polyoxyehtylene esters of fatty acids, polyoxyethylene sorbitol esters (Tween) and lipids. Negatively charged surfactants include (but not restricted to) di-(2-ethyl-hexyl) sodium sulfosuccinate (AOT), sodium dodecylsuphate (SDS), sodium dodecylsuphonate , and sodium dodecyl-N-sarcosinate. The zwitterionic surfactant could contain anionic and cationic groups on the alpha and omega positions of a long aliphatic chain. For zwitterionic surfactants that contain both anionic and cationic groups on the alpha and omega positions of a long aliphatic chain, complex formation should be done under acidic conditions so that the surfactant can have a positive charge that will interact with the nucleic acid. The anionic portion is neutralized by being protonated and therefore interacts with the non-aqueous phase. After formation of the complexes, the complexes are extracted into an aqueous solution containing a higher pH than the pH used to form the complexes.

Brief Description of the Drawings

FIG. 1. Circular dichroism spectra measured for samples of plasmid DNA added to a mixture of Brij30/TMP or DNA alone at 30°C. The ellipticity value for control samples prepared without DNA were subtracted from the experimental samples.

Detailed Description

A complex is described that is deliverable to a cell comprising inserting a nucleic acid or other cargo into a reverse micelle. The reverse micelle has the property to compact the nucleic acid for easier delivery. The term deliverable means that the complex is capable of being delivered as defined in this specification.

A process for forming a negatively-charged, zwitterionic, or neutral complex for delivery to a cell, comprising forming a cationic reverse micelle using amphipathic molecules. Then inserting a biologically active compound into the cationic reverse micelle.

Analysis:

Two types of micelles appear to be present in the samples. There are small, "empty" micelles, and large pDNA containing micelles. It appears that the size of micelles containing pDNA increases as the concentration of pDNA increases. The micelle appears to be saturated at a size of 50-60 nm.

Example 5: Conformation of PCILuc DNA in Inverse Micelles.

Procedure:

pDNA (60 μg) was taken up in 10 mM potassium phosphate buffer at pH 7.5 (20 μL and 60 μL). The pDNA solutions were added to a mixture of Brij 30/TMP (1 mL, 1:7.3 v/v) and agitated (2 min). The circular dichroism spectra were measured for each sample (cell length = 0.5 cm, Spectropolarimeter 62DS, Avive Associates) at 30°C against control samples prepared without the pDNA (FIG. 1, the ellipticity value for the control samples were subtracted from the experimental samples).

Results:

There are shifts in the position of both the positive and negative bands and in the position of the cross-over point for the 20 μL pDNA solution (W0=3.35). Spectra that are similarly shifted are broadly defined as -spectra, and are attributed to a condensed form of pDNA. In contrast the spectra of the 60 μL pDNA solution (W0=10.05) resembles the spectra of DNA in buffer alone in respect to cross-over point. However this spectra is characterized by an increase in the intensity of the negative band (maximum at 240 nm).

Example 6: PCILuc DNA Condensation.

Part A. Ethidium Bromide.

Procedure:

A solution of pDNA in HEPES (25 mM, pH 7.8) and EDTA (0.5 mM) (3-67 μL) containing ethidium bromide (0.9 μg, Sigma Chemical Company) was added to a mixture of Brij 30/TMP (0.7 mL, 1:7.3 v/v) and agitated. After 4 h at ambient temperature, the samples were assayed utilizing a fluorescence spectrophotometer (Hitachi, Model F-3010), with an excitation wavelength of 525 nm and an emission wavelength of 595 nm.

Results:

Volume (μL)	W0	I/Imax*100
3	0.72	15